REMARKS

Upon entry of the above amendment, claims 21, 33, 43, and 45-53 will be pending, claims 19, 20, 22, 31, 32, 34, and 44 having been newly canceled without prejudice and new claims 45-53 added. The amendments to claim 21 are supported by original claim 9, by claim 22 (now canceled), and by the specification at page 5, lines 2-6; and page 10, line 32, to page 12, line 21. The amendments to claim 33 are supported by original claim 9, by claim 34 (now canceled), and by the specification at page 5, lines 2-6; and page 10, line 32, to page 12, line 21. The amendments to claim 43 are supported by claim 44 (now canceled), and by the specification at page 6, lines 19-29; and page 12, lines 11-17. New claims 45-53 are supported by the disclosure at page 11, lines 1-6, and at page 18, lines 20-26, each of which shows a linker sequence that contains four repeats of the 5-mer GlyGlyGlyGlySer, each repeat encoded by 15 base pairs. The new claims are further supported by the disclosure at page 18, lines 28-35, which illustrates reduction of the long linker of 20 amino acids (encoded by 60 base pairs) to a short linker containing the 5-mer GlyGlyGlyGlySer. Also see page 12, lines 15-17, which says that the long linker sequence is preferably encoded by 45 to 60 base pairs (which would correspond to three to four repeats of the GlyGlyGlyGlySer sequence). No new matter has been added.

Applicants thank the Examiner for acknowledging that the prior rejections for lack of enablement and for obviousness in view of McGuinness et al. and Volkel have been withdrawn. The present Office action maintains the rejection for lack of written description and asserts a new ground of rejection for obviousness. These rejections are addressed in turn below.

Rejection for lack of written description under 35 USC § 112, ¶ 1

Claims 19-22, 31-34, 43, and 44 were rejected as failing to comply with the written description requirement. Applicants disagree with the grounds for the rejection as set forth in the Office action, but in an effort to move this application to allowance have canceled claims 19, 20, 22, 31, 32, 34, and 44, and amended the remaining claims. Reconsideration of the rejection as it may be applied to the presently pending claims is respectfully requested.

The Office action asserts that the claims lack written description support because the specification allegedly does not sufficiently describe the linkers used in the claimed methods. According to the Office action at page 6, "Applicants have not demonstrated a structure/function correlation for the genus of claimed linkers that would allow the proper translation and folding of each scFv in the diabody in order to bind the respective target antigens." Applicants point out once again that the function of the peptide linkers used in single chain antibodies depends on the length of the linker, but not on the sequence. As described in the specification, and as well known in the art, relatively long linkers (e.g., encoded by 30-150 base pairs) possess the flexibility needed to fold and thus allow the two linked variable domains to associate with each other. In contrast, a relatively short linker (e.g., encoded by fewer than 27 base pairs) is too short and inflexible to form the bend that permits the variable domains on either side of the linker to come into contact with each other. When a short linker is present between a light and heavy chain variable domain, those two variable domains are available for association with other heavy and light chain variable domains located elsewhere on the same or a different polypeptide chain. Thus, the "structure/function correlation" for antibody linkers such as are useful in the presently claimed methods is well known in the art, and need not be "demonstrated" by Applicants.

Applicants understand that some of the Examiner's concerns seem to be based on the method as described in claims 19 and 31, which required only one restriction site within the first nucleotide linker of 30-150 base pairs, and so permitted a large range of linker lengths after treatment with of that first linker with the restriction enzyme and ligation. Claims 19 and 31 are presently canceled. Claims 21 and 33 are amended to specify that the first and second nucleotide linkers are 45 to 60 base pairs in length, and further to specify that the two restriction sites in the first nucleotide linker are located such that treatment with the two restriction enzymes and subsequent ligation result in a pair of linkers, each encoded by 6 to 27 base pairs. Thus, the length of each of the two "short" linkers present in the ultimate construct is now limited to 6 to 27 base pairs, corresponding to 2 to 9 amino acids. The claims no longer cover use of a first nucleotide linker with the restriction site(s) located "just anywhere in the linker" (see Office action at page 8. Volkel et al., Protein Engineering 14(10):815-823 (2001), demonstrated that,

while the linker length was important for function, the sequence of amino acid residues in the linker was not. Applicants submit that, because the length of the linker is the only important variable in defining how it functions, the presently claimed methods would be expected to produce single chain diabodies that function appropriately. It is clear that Applicants were in possession of, and fully described, the methods as presently claimed. Withdrawal of the rejection is respectfully requested.

Rejection for obviousness under 35 USC § 103(a)

Claims 19-22 and 31-34 are rejected as obvious over Little et al. (CA 2331641 or DE19819846) in view of McGuinness et al. and Volkel (already of record). Claims 19, 20, 22, 31, 32, and 34 have been canceled, so the rejection is moot as to them. To the extent the rejection may apply to claims 21 and 33 as amended (or to new claims 45-50, which depend from claims 21 and 33), Applicants respectfully traverse.

The Office points in particular to Figure 2 of Little, characterizing it as showing "restriction digestion of a first linker in a first scfv; restriction digestion of a second linker in a second scfv and recombining the VH1-VL2 and the VH2-VL1 constructs into a single fused scfv diabody construct for producing libraries." See page 10 of the Office action. Applicants submit that this is a mischaracterization of what Figure 2 actually shows.

The experiments illustrated in Figure 2 are described in Example 1 of Little et al., beginning at page 10 of this reference. Little et al. started with two scFv constructs and employed PCR to introduce the desired linkers in the final constructs. See, for example, the steps labeled "PCR 1" and "PCR 2" in the upper left corner of the figure and the step labeled "PCR 3" in the middle right portion of the figure. PCR 1 was used to sequester a heavy chain variable domain (VH19) from its corresponding light chain variable domain and to add a linker and EcoRV restriction site adjacent to VH19; this variable domain/linker was then ligated with a light chain variable domain, VL3, from a different antibody, to produce an intermediate construct (pHOG19-3) containing VH19, L1 (a short linker), and VL3. In a separate step, PCR 2 was used to sequester VL19 from its corresponding VH19, and to introduce a linker and restriction site

adjacent to VL19. This PCR 2 step was followed by ligation with a heavy chain variable domain, VH3, to produce a second intermediate construct (pHOG3-19) containing VH3, L1 and VL19. Then the pHOG3-19 construct was again subjected to PCR ("PCR 3") to introduce yet another linker sequence, resulting in yet another intermediate construct. This last intermediate construct was then ligated into pHOG19-3 upstream of VH19-L1-VL3. The final construct, pDISC3x19-LL, contained VH3- L1- VL19- L2- VH19- L1-VL3, with L1 being a short linker and L2 a long linker. Although restriction enzymes were employed in the course of generating pDISC3x19-LL, at no point was a restriction enzyme used done to split a long linker into two short linkers that were subsequently ligated to the ends of a VH-long linker-VL or VL-long linker-VH construct, as required by claims 21 and 33.

In marked contrast to Little et al.'s scheme, the presently claimed method does not need to utilize PCR to introduce any of the three linker sequences, all of which are present in the starting constructs. The presently claimed method begins with two scFv constructs (or two scFv libraries), the first of which has cleavage sites for two restriction enzymes strategically located within its long linker and the second of which has cleavage sites for the same two restriction enzymes at the ends of the VH- and VL-encoding sequences distal to the long linker. Cleavage of the first construct (or library) with the two restriction enzymes opens up the first scFv coding sequence by cutting in the long linker, leaving a short linker sequence attached to the sequence encoding the VH and another short linker sequence attached to the sequence encoding the VL. The second construct (or second scFv library) is treated with the same two restriction enzymes to prepare the ends for ligation, and then ligated between the two short linker sequences of the first construct. This efficiently produces the final construct, with no need to use PCR to add linkers or other sequence and no need for the multiple manipulations of Little et al. Further, unlike the Little et al. scheme, which initially separates the two pairs of variable domains into four different constructs and then reassembles them into two and finally one construct, Applicant's methods split only one of the two pairs, leaving the other pair of variable domains attached to each other. Except for the fact that both ultimately produce a single chain diabody construct, Applicants submit that Little et al.'s scheme bears no resemblance to the presently claimed methods.

Further, Applicants see no evidence that Little et al. intended their scheme for the purpose of "producing libraries", as asserted by the Office action. Applicants ask the Examiner to point out where in Little et al. she sees such disclosure. Given the sequence-specific nature of the PCR steps, Applicants fail to see how Little et al.'s laborious method could be employed in the context of a library.

The McGuinness reference is addressed at pages 10-12 of the Office action. According to the Office action at page 11, "McGuinness teaches a diabody format: VHA-VLB-rbs (linker)-VHB-VLA which reads on the instant claims." This is simply wrong, as was pointed out by Applicants in the Reply filed January 7, 2008, again in the telephonic interview with the Examiner and Applicants' undersigned representative on August 28, 2008, and yet again in the Reply filed subsequent to that interview, on January 13, 2009. McGuinness does not teach anything that reads on the instant claims. The disclosure in McGuinness to which the Office action refers describes preparation of a diabody construct "**ribosome binding site (rbs)-Leader 1-VHA-VLB-Stop-rbs-Leader 2-VHB-VLA-His6-Myc-Amber codon-fd gene 3**." (See page 1150, right column; also see Figure 1 on page 1150.) The Examiner apparently believes that the term "Stop-rbs-Leader" is equivalent to a sequence encoding a linker. It is not. "Stop" means stop codon—i.e., the end of translation. "Rbs" means ribosome binding site—i.e., a site where a ribosome can attach in order to start translation. "Leader" means a leader peptide—i.e., a peptide that occurs at the amino terminus of a polypeptide. It is clear that the construct described by McGuinness codes for expression of two entirely separate polypeptides, with two ribosome binding sites, two leader peptides, and a stop codon between the two coding sequences. McGuinness sought to design a construct that encodes two separate scFv, putting both coding sequences on a single construct so that they would both be expressed in the same cell and could assemble as a two-chain diabody. See, e.g., the discussion at page 1149, right column, about use of diabodies ("Diabodies are dimeric antibody fragments formed by cross-pairing two scFv molecules.") and at page 1152, right column, stating that "each clone produces two polypeptide chains." McGuinness did not disclose a construct that encodes two VH and two VL in a single polypeptide, as required by the presently claimed methods. Since this has been pointed out to the

Examiner repeatedly over the course of this prosecution, including during the interview of August 28, 2008, and the Examiner at that time acknowledged that Applicants' view was correct, Applicants are at a loss as to why it was raised yet again in the present Office action. McGuinness did not disclose use of a first antibody library in which the sequences encoding the heavy and light chain variable domains are linked by a nucleotide linker containing two restriction sites, as required by step (a) of claim 21, and particularly where those two restriction sites are located in accordance with step (e). It follows that this reference also does not disclose a step of cleaving at those restriction sites, as required by step (c). Furthermore, MGuinness does not disclose ligating a sequence encoding a second pair of heavy and light chain variable domains to those cleaved restriction sites, thereby producing a construct encoding a single polypeptide containing two heavy and two light variable domains, all linked by linkers of the lengths defined in claim 21. In fact, it is difficult to derive from McGuinness any teaching of relevance to the presently claimed invention. Applicant is puzzled as to why the Examiner continues to cite McGuinness and continues to mischaracterize its teachings, despite the ample evidence of record that this reference is irrelevant.

Unlike McGuinness, Völkel does disclose constructs encoding single-chain diabodies. However, any similarity to applicant's library methods pretty much ends there. Völkel started with a known single chain diabody construct containing four variable domains (pAB1 scDb CEAGal), not a pair of libraries, each member of which encodes two variable domains, as required by steps (a) and (b) of claim 21. Thus, none of the subsequent steps of the claim, all of which require manipulations of those libraries, was carried out by Völkel. As Völkel was interested in determining the effect of linker length on function of the diabody, Völkel used several restriction enzyme sites located in the pAB1 scDb CEAGal construct's three linkers as a means to generate a library of constructs that varied solely in the lengths of the three linkers (page 816, columns 1-2; fig.2). Völkel did not ligate a nucleic acid fragment encoding two variable domains into a pair of cleaved restriction sites between two variable domains, as required by step (e) of claim 21, and provided no reason to contemplate doing so. (In fact, introducing extra variable domains into her construct in that manner would have ruined her

experiment, the purpose of which was simply to study the effect of linker length.) Accordingly, Völkel does not disclose any of the steps of the present claims. Since Völkel was not concerned with combining two libraries, each encoding two-variable-domain single chain antibodies, to prepare a four-variable-domain, single chain diabody library, and in fact started out with a construct that already contained all four variable domains in a single chain, Völkel provides no incentive to make the considerable modifications that would be required to result in a method such as applicant's. Völkel certainly does not supply what is absent from McGuinness, and vice versa.

It is not at all clear to Applicants how the Examiner views the three cited references as together rendering the present claims obvious. According to the Office action at pages 12-13,

> One skilled in the art would have been motivated and been assured of reasonable success in having produced the instant methods at the time of the invention based on the combined disclosures of Little McGuinness and Volkel because each disclose the technology for constructing single-chain diabody phage display libraries where a scFv recognizing a first antigen comprising a linker with a restriction enzyme site and a second scFv recognizing a second antigen comprising a linker are treated with a restriction enzyme in order to obtain fragments which are then ligated in order to construct a final fragment having the VH and VL domains against the second antigen inserted between the VH and VL domains against the first antigen are assembled into the diabody phage display library. Each of the references discloses techniques involving differential restriction enzyme digestion of various fragments and the technology for selective insertion of the VH2/VL2 or VL2/VH2 pair between the VH1/VL1 or VL1/VH1 domains to generate a phage display diabody library. (Informal English in the original.)

The Office action's summary of what each of the three references discloses is simply inaccurate on several points.

Contrary to the quoted statement, Little says nothing about constructing a phage display library or a single-chain diabody library. In fact, Little did not discuss libraries at all.

Contrary to the quoted statement, Little did not treat two scFv with a restriction enzyme. While Little did treat one scFv (pHOG-dmOKT3) with a restriction enzyme, Little's second scFv (pHOG-αCD19) was disassembled not by use of a restriction enzyme, but rather by using a PCR step to sequester the VH from its VL and introduce a linker and restriction site adjacent to the VH, and a second PCR step to do the same for the corresponding VL. (If the Examiner was

referring to the intermediate construct pHOG19-3 as the "second scFv recognizing a second antigen," Applicants note that pHOG19-3 contained unrelated VH and VL, so was unlikely to recognize a second antigen.)

Contrary to the quoted statement, McGuinness did not disclose technology for constructing a single-chain diabody library. Rather, McGuinness disclosed methods for making a library, each member of which encodes two separately expressed scFv that could associate after expression into a non-covalently linked, two-chain diabody.

Contrary to the quoted statement, Volkel did not disclose treating two scFv with a restriction enzyme (or at least Applicants cannot find such disclosure). Volkel started with a construct that encoded all four variable domains as a single polypeptide (a "single-chain diabody"), rather than working with the two separate scFv.

Contrary to the quoted statement, Volkel did not disclose technology for selective insertion of one pair of variable domains between a second pair of variable domains. Such a method would have been useless for Volkel's experiments, since she started with a construct encoding a single-chain diabody in which the four variable domains were already together in a single polypeptide.

None of the cited references could have disclosed anything like the method of claim 21 because no one in the art realized the significant advantage of starting with two specially-designed scFv libraries, one with two restriction sites placed in particular places in the linker sequence and the other with matching restriction sites placed at the outer edges of the variable domains (and with a long linker between the variable domains). By starting with those two specially-designed libraries, one can readily, with minimal manipulations, create a single-chain diabody library with three appropriately sized linkers. Further, the single-chain diabody library created by this method has the significant advantage of maintaining the VH-VL pairing of at least one of the scFvs (the pair that ends up in the middle of the construct). This means that each member of the constructed single chain diabody library necessarily has at least one functional VH-VL pair, which makes subsequent screening for activity simpler. The cited art does not even hint at this beneficial effect of the presently claimed method, much less describe a way to accomplish it.

The Examiner has not begun to meet her burden of explaining why it would have been obvious to (1) start with one of the cited disclosures and (2) make the modifications necessary to result in the presently claimed methods. Further, she has not accurately characterized the disclosures of the references in comparison with the presently claimed methods, so it is not clear where in those references one of ordinary skill would have found a starting point, much less why one would have modified the prior art method to arrive at the claimed methods. Merely citing three references that are vaguely in the same field as the claimed methods and discussing what those references supposedly disclose is insufficient, even if had been done accurately. Nor is it sufficient to rest an obviousness rejection on the fact that general techniques (such as restriction enzyme digestion) employed in very particular ways in the claimed method have been generally used in the art, where the art provides no clue that the techniques should be used as claimed. The Office action liberally quotes from the Supreme Court's decision in *KSR v. Teleflex*, but omits the most pertinent quote of all: "[**A court should] determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue. To facilitate review, this analysis should be made explicit. 'Rejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness**.'" (127 S.Ct 1741; internal citation omitted) The U.S. Patent and Trademark Office issued a memorandum to examiners on May 3, 2007, explicitly reminding examiners that *KSR* did not alter the requirement that an examiner formulating a rejection under § 103(a) identify the reason why a person of ordinary skill in the art would have combined the prior art elements in the manner claimed. This has not been done in the present case. If the Examiner intends to maintain the rejection, she is asked to provide the requisite articulated reasoning as to why she believes one of ordinary skill would start with what is actually described in one of the references and make the precise modifications necessary to result in the claimed method, so that Applicants can respond. Absent such a showing, Applicants submit that the rejection should be withdrawn and the claims allowed.

Conclusions

Applicants believe that all claims are now in condition for allowance, and such action is respectfully requested.

Filed with this response is an Information Disclosure Statement, a Petition for Extension of Time, and the requisite fees for both. Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

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